AWARD NUMBER: W81XWH-10-1-1036

TITLE: *Simulation of Blast Loading on an Ultrastructurally-based Computational Model of the Ocular Lens*

PRINCIPAL INVESTIGATOR: Richard A. Regueiro, Ph.D.

CONTRACTING ORGANIZATION: Regents of the University of Colorado
Boulder, CO 80309-0001

REPORT DATE: October 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
October 2014	Annual	30Sep2013 - 29Sep2014

4. TITLE AND SUBTITLE
Simulation of Blast Loading on an Ultrastructurally-Based Computational Model of the Ocular Lens

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-1036

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Richard A. Regueiro

richard.regueiro@colorado.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Regents of the University of Colorado
3100 Marine St., 572 UCB
Boulder, CO 80309-0001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Research progress over the past year focused on imaging of lens fiber cell structure using confocal laser scanning microscopy (CLSM), and type IV collagen structure using cryo-electron microscopy and tomography (cryo-EM and ET). The CLSM has demonstrated the ability to generate good structural identification of lens fiber cells at 10X objective, while the cryo-EM and ET have not yet positively identified type IV collagen structure of the lens capsule. The collaborations with Dr. Vernerey of CU Boulder on large deformation multiscale Lagrangian-Eulerian computational modeling of the whole lens, and Dr. Burd at the University of Oxford on multiscale modeling of the lens capsule, will lead to positive steps toward developing a multiscale model of the ocular lens.

15. SUBJECT TERMS
lens capsule ultrastructure, lens fiber cell ultrastructure, multiscale modeling of lens capsule mechanics

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	17	19b. TELEPHONE NUMBER *(include area code)*

Contents

1 Introduction

In the life of a combat soldier, traumatic cataract in ocular lenses may result from blast loading, whereby (i) the lens capsule (Fig.1) is perforated by intraocular foreign bodies (IOFBs [Walter, 1962, Mader et al., 1993, Parver et al., 1993, Wong et al., 1997, Mader et al., 2006, Weichel and Colyer, 2008]) which in turn damage the lens fiber cells, (ii) the lens is loaded fluid dynamically by the surrounding aqueous and vitreous humors [Banitt et al., 2009] (see Fig.1), and/or (iii) the lens internal substance (crystallins lens fiber cells) is stressed by the passing shock wave. Traumatic cataract can result in a partially or fully clouded lens, complete dislocation of the lens (floating between aqueous and vitreous humors, see Fig.1), or zonule rupture such that partial or full vision loss may occur. The mechanisms of traumatic cataract formation that may require cataract surgery (implantation of an intraocular lens (IOL)) are not well understood in comparison to the mature and ever-improving surgical technology and procedures. The **hypothesis of the research** is that an ultrastructurally-based computational finite element model of the ocular lens subjected to blast loading can assist in better understanding how traumatic cataract is formed in the combat soldier, and in turn improve our understanding of traumatic cataract in civilians whose eyes are subjected to impact loading. The **scope of the research** is to develop a multiscale, ultrastructurally-based, computational model of the ocular lens subjected to blast loading, in conjunction with imaging methods to identify lens capsule and internal substance structure and mechanical experiments for calibrating material model parameters.

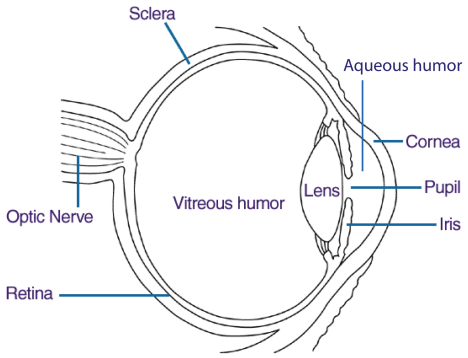

Figure 1. Eye cross-section. `www.nei.nih.gov`.

2 Body

The research tasks focussed on over the past year have been the imaging ones (Task 6), and multiscale computational modeling (Task 1-3). For Task 6, undergraduate students Sai and Sri Radhakrishnan have taken the lead on the cryo-electron microscopy and tomography (cryo-EM and ET) (Sai) imaging of type IV collagen structure, and confocal laser scanning microscopy (CLSM) (Sri) for the internal lens fiber cells. A multiscale computational model of the lens capsule, within a combined Eulerian-Lagrangian approach to large deformational loading of the lens, and solid-fluid interaction, is being pursued in collaboration with Assoc. Prof. Franck Vernerey and his recently-graduated PhD student, Dr. Louis Foucard.

A No Cost Extension (NCE) has been recently filed, to extend the project through September 2016.

Next, research on the Tasks over the past year are summarized.

6a. Before and after whole porcine lens unconfined compression, image lens fiber cell geometry using CLSM (low strain rate subtask 4a: months 1-4; higher strain rate subtask 4b: months 6-8).

In collaboration with Dr. Christopher English of the Molecular, Cellular, and Developmental Biology (MCDB) department, we have obtained reasonable resolution images of cross sections of porcine lens fiber cell structure, such that numerical geometric models (a.k.a., "meshes") can be determined based on these images. More details are provided in the Senior Thesis (*Ultrastructural Identification of the Internal Fiber Cells of the Mammalian Ocular Lens*) by Ms. Srinidhi Radhakrishnan, who graduated in May 2014 from the Department of Chemical and Biological Engineering at UCB; see attached for Senior Thesis as PDF.

It was determined that fiber cells imaged with 10× objective (see Figs.2,3) produced enough resolution to discern the lens fiber cell structure, whereas images obtained with 5× objective (see Figs.4,5,6) did not provide enough resolution, essentially rendering all fiber cell walls to appear straight (Fig.6) instead of having waviness to them (Fig.3). As was demonstrated in C. Bay's MS Thesis (see 2nd Year Annual Report, and Fig.7), 40× objective could discern the hexagonal cross-sectional pattern of the lens fiber cells in porcine lenses, but such resolution is too high to reconstruct a whole structural image/geometrical model of the lens fiber cells (see Fig.2 for one full through-thickness slice using 10× objective, and Fig.3 for a zoomed region).

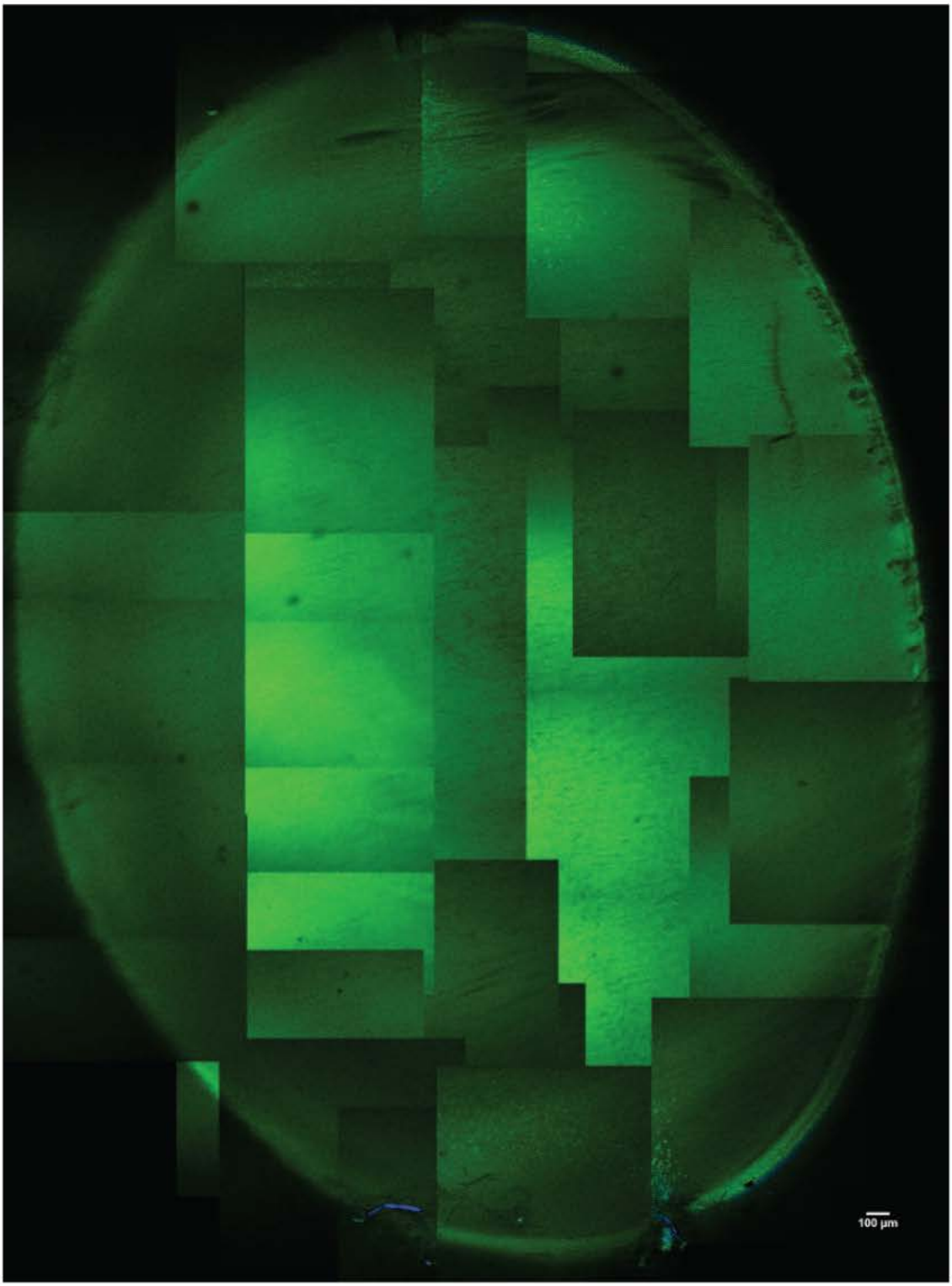

Figure 2. Full through-thickness CLSM slice of porcine lens fiber cell structure at 10X objective.

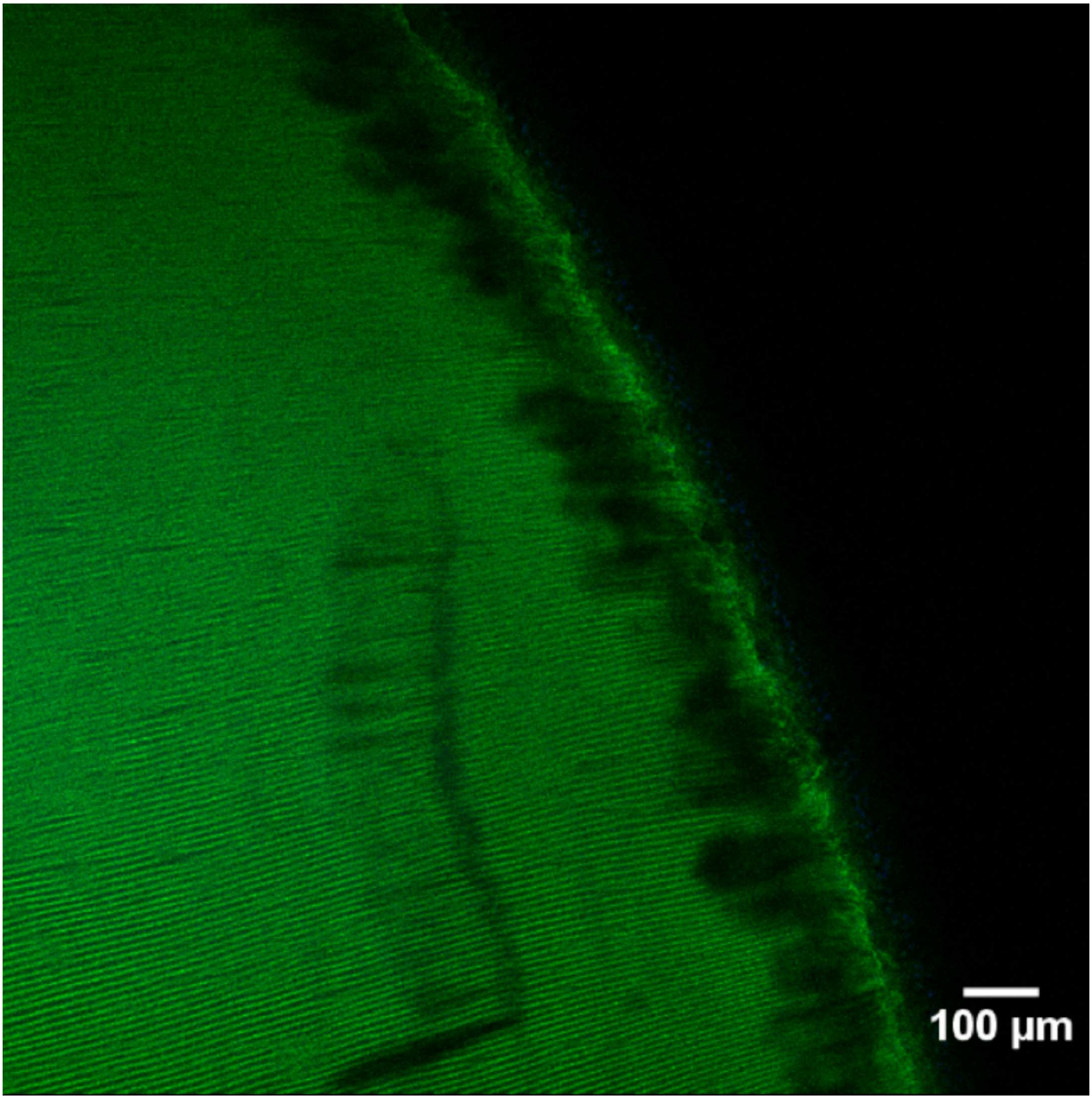

Figure 3. Zoom-in on upper right region of CLSM slice (in Fig.2) of porcine lens fiber cell structure at 10X objective.

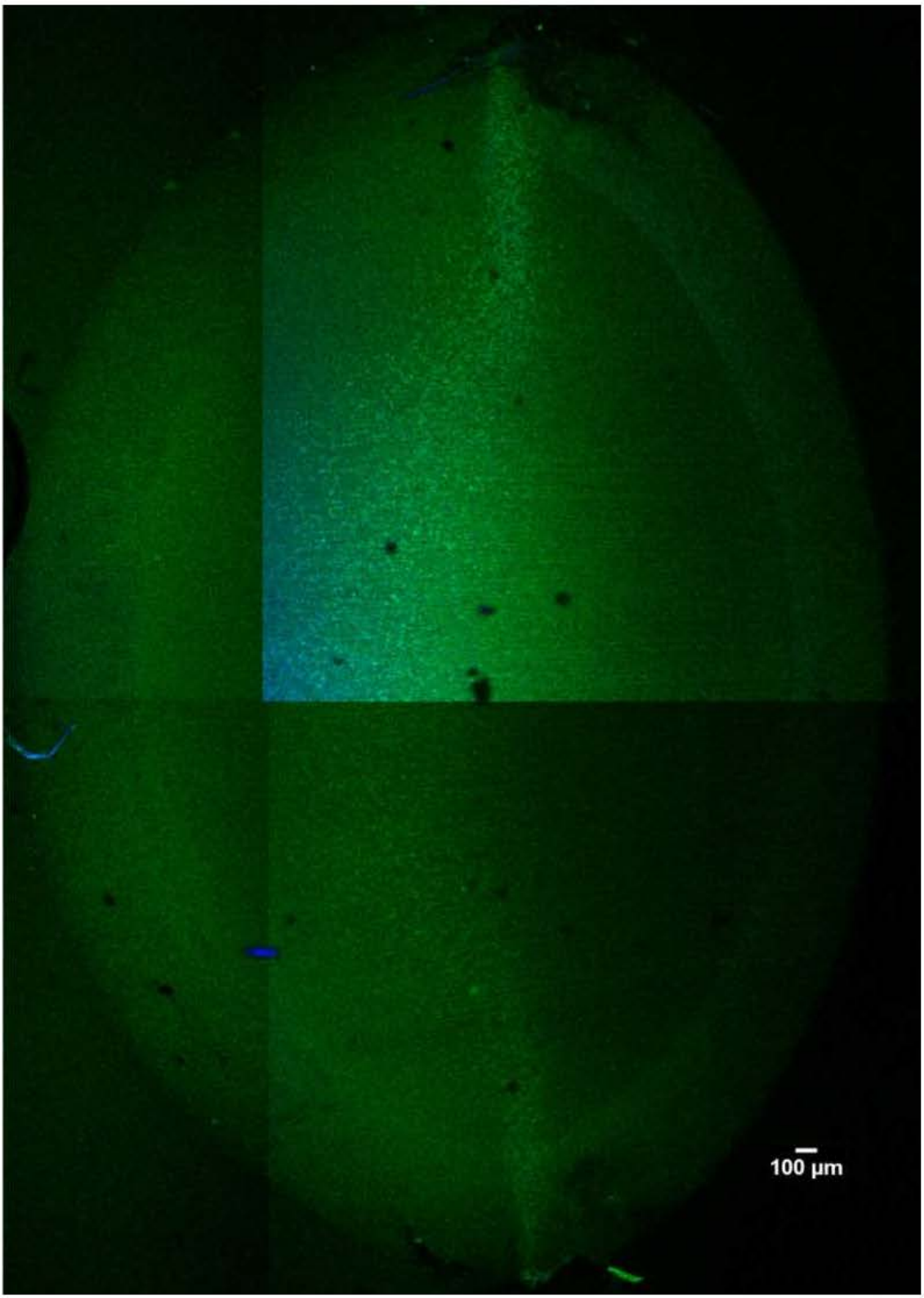

Figure 4. Full through-thickness CLSM slice of porcine lens fiber cell structure at 5X objective.

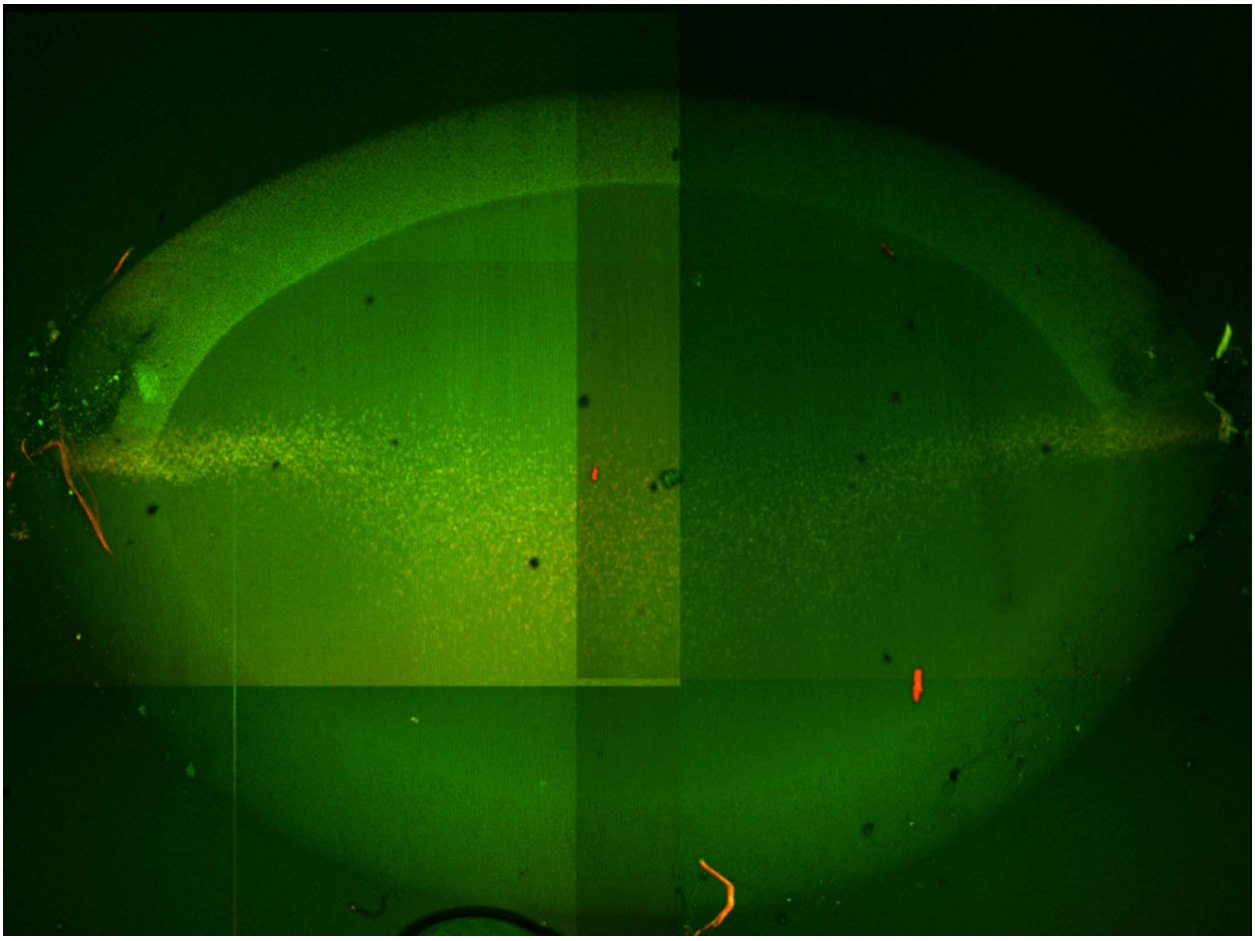

Figure 5. Full through-thickness CLSM slice of porcine lens fiber cell structure at 5X objective.

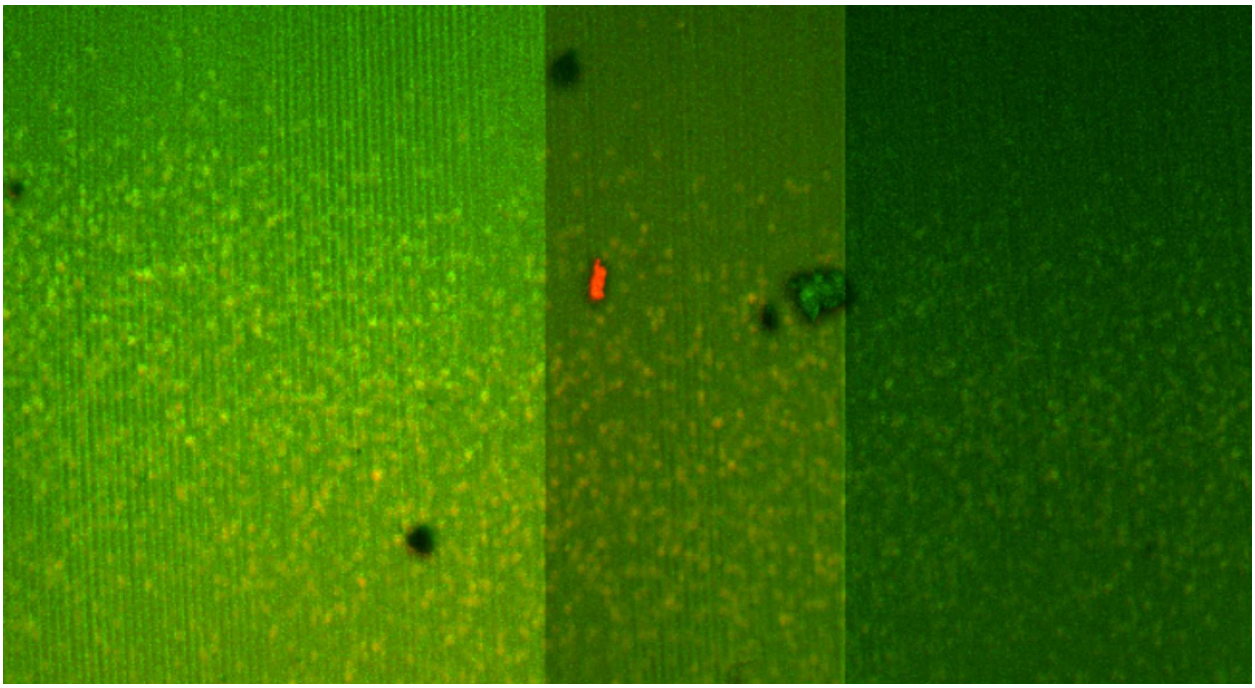

Figure 6. Zoom-in on CLSM slice of porcine lens fiber cell structure at 5X objective (refer to red dot in center of image in Fig.5).

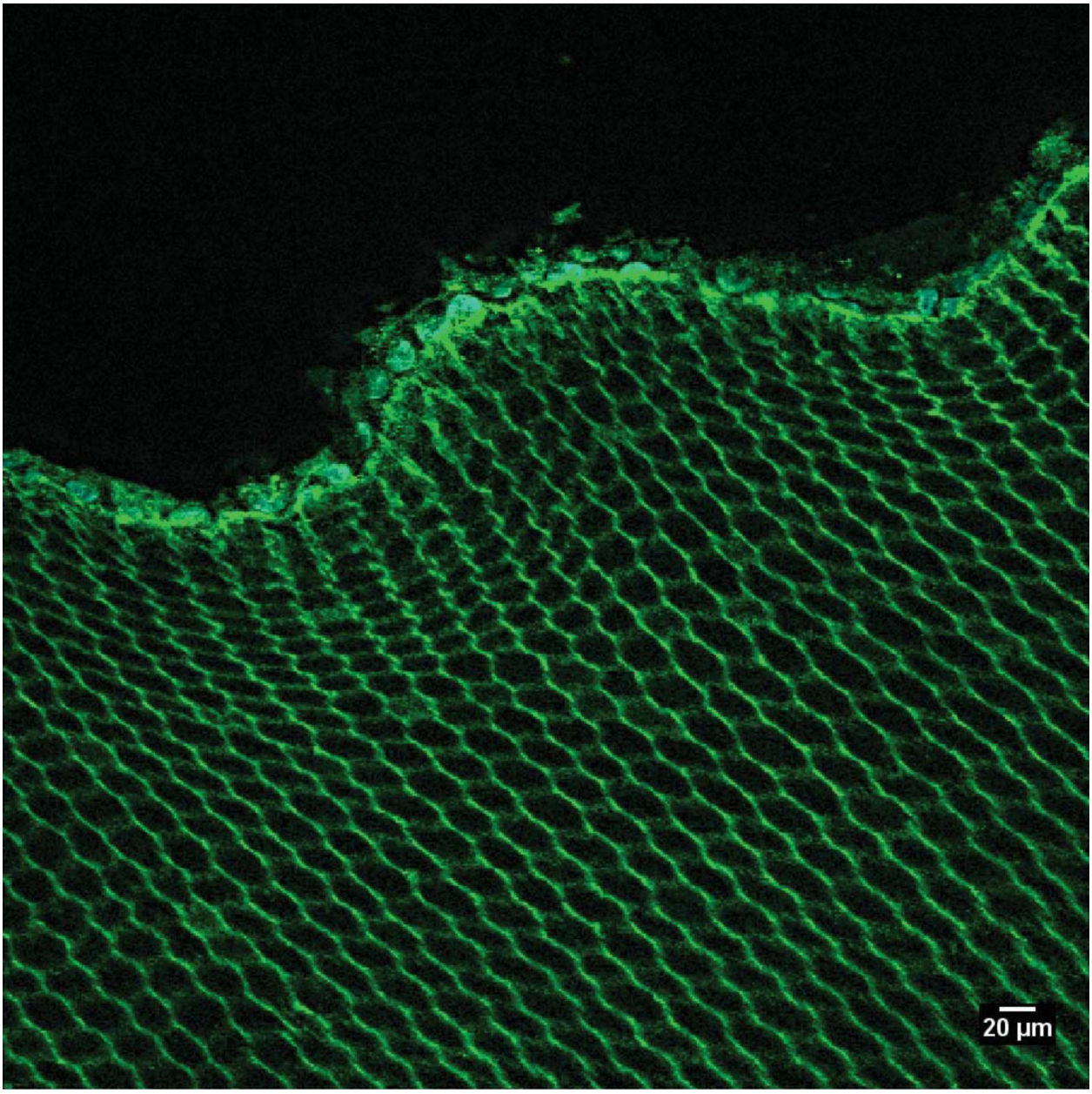

Figure 7. CLSM slice of porcine lens fiber cell structure at 40X objective.

6b. On as-received porcine lens capsules, image type IV collagen ultrastructure in lens capsule using cyro-electron tomography (CryoET) (months 1-4).

The debate has been settled within the Department of Molecular, Cellular, and Developmental Biology (MCDB) at the University of Colorado Boulder (UCB) as to whether the structural images we are obtaining of the type IV collagen meshwork of the lens capsule via CryoET are in fact an artifact of the pressurized freezing process, or actual ultrastructure of the tissue. It is believed that the observed "structure" in Fig.8, for instance, is artifact. Procedures to etch the proteins away, except the type IV collagen, are being investigated anew. Initially, the CryoET was preferred because it would preserve the native structure as much as possible, but then the process also preserves the state of other proteins as well. The more invasive procedures that attempt to wash away all proteins except type IV collagen are being considered. Also, shadowing techniques like that used in Barnard et al. [1992] are being considered to see if such images can be replicated. Such a machine exists at UCB in the MCDB department, but it is currently not working. This research is continuing.

Furthermore, a new PhD student in the Biomedical Engineering program within the Department of Mechanical Engineering at UCB, Ms. Julia Taussig, has been recruited to continue the imaging research (Task 6), as well as nanoindentation on lens capsules (Task 5), during the 2 year extension.

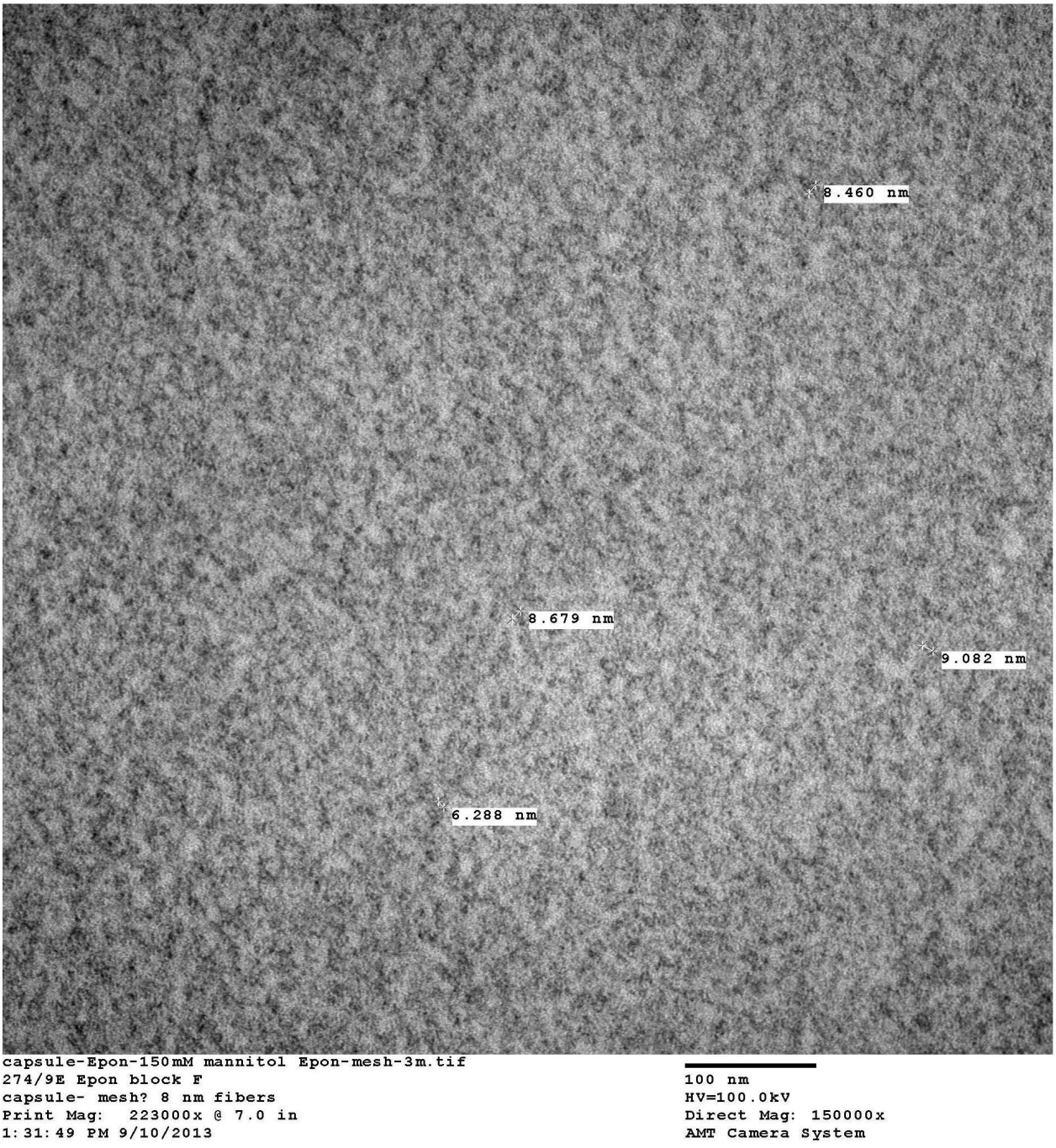

Figure 8. Cryo EM image of what is possibly type IV collagen structure. It has been determined by MCDB at UCB that what appears to be structure is in fact an artifact of the freeze-substitution process.

2c: Formulation and finite element implementation of multiscale perforating finite strain biphasic mixture (solid and fluid) solid-shell continuum model of lens capsule in Tahoe, to model cutting/ perforation of lens capsule based on implementation in subtasks 1c and 2b (months 28-36). 3b: Using result of subtask 3a, formulate and implement multiscale hierarchical, anisotropic, lens fiber cell equivalent soft viscoelastic constitutive model of the internal lens substance (months 40- 48).

For the computational modeling, we are continuing to work on a large deformation hybrid Lagrangian-Eulerian simulation of the lens puncture tests in order to eventually model penetration by Intra-Ocular Foreign Bodies (IOFBs), but also shock propagation and solid-fluid interaction between the lens and vitreous and aqueous humors. This is in collaboration with Assoc. Prof. Franck Vernerey, and his recently graduated PhD student Dr. Louis Foucard, at UCB.

The current status of the research is a preliminary attempt to relate the type IV collagen ultrastructure to the macroscale lens capsule mechanical response. Assumptions include (1) in-plane stretching of two-dimensional type IV collagen meshwork (rather than actual 3D structure and out-of-plane loading, not yet identified by CryoET; similar assumption made by Burd [2009]), (2) bending allowed at fibril junctions (better than assuming pinned junctions, such as in Burd [2009]), and (3) periodic boundary conditions (appropriate for large deformation under indentation, but not tearing/puncture). An illustration of the concept and preliminary results compared to the Krag and Andreassen [1996] data, and Burd's fit of the Krag data, and the fit provided by our model, are shown in Fig.9.

In addition, PI Regueiro is currently spending his sabbatical in Autumn 2014 at the University of Oxford collaborating with Dr. Harvey Burd on multiscale finite element modeling of the lens capsule.

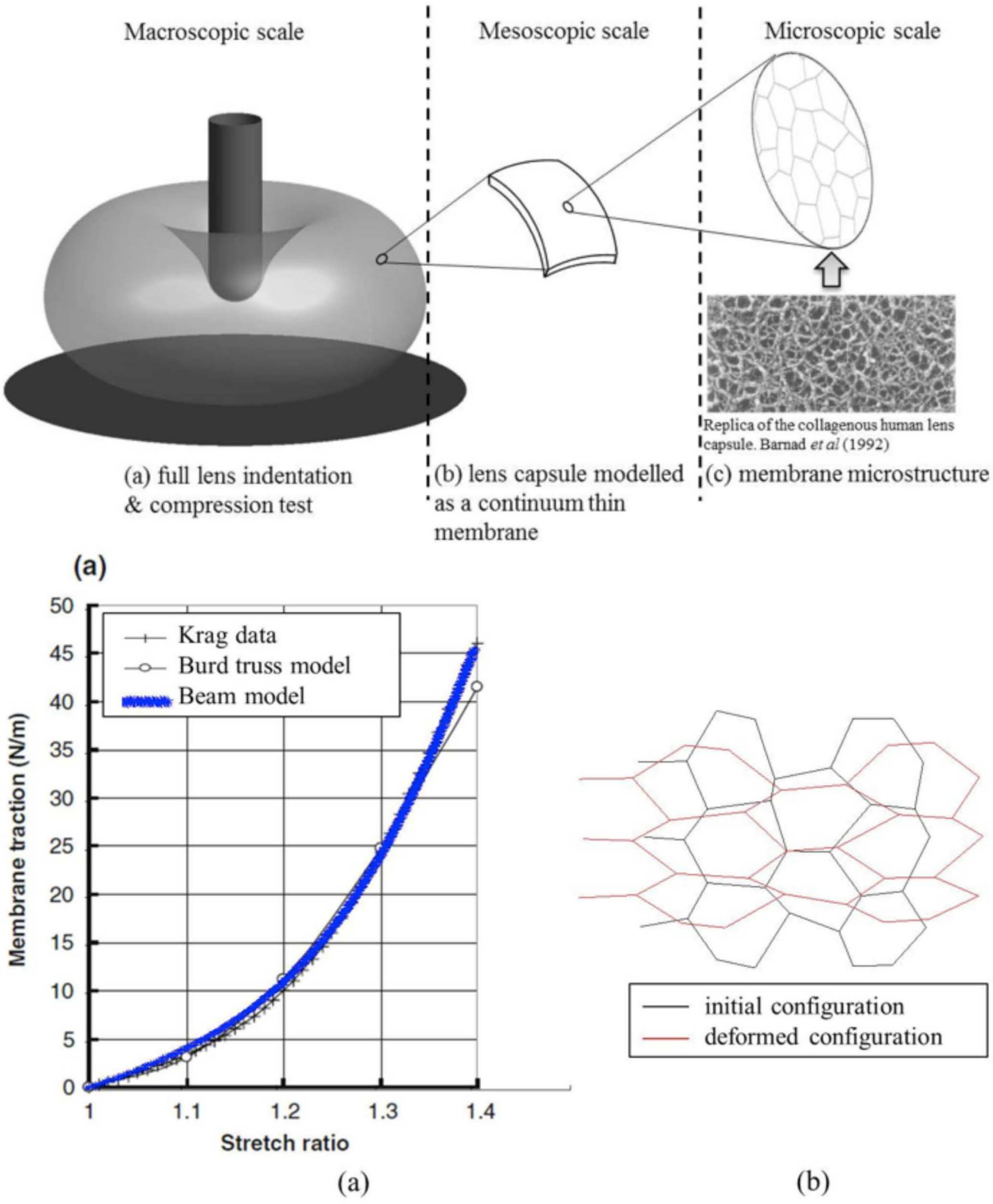

Figure 9. (top) Concept of multiscale modeling of lens capsule, showing network approximation based on ultrastructural imaging [Barnard et al., 1992]. (bottom) Comparison of traction versus stretch ratio of lens capsule experimental data [Krag and Andreassen, 1996], other truss network model [Burd, 2009], and our beam model.

3 Key Research Accomplishments

- CLSM images of the internal lens fiber cell structure have successfully reconstructed one through-thickness slice at 10× objective for a porcine lens, with discernable fiber cell wall geometry.
- The MCDB department and Bio3D lab `bio3d.colorado.edu` at UCB have determined that the Cryo-EM/ET techniques used thus far have generated images which only show "artifact structures" of the freeze-substitution process, and not actual type IV collagen ultrastructure.
- A preliminary network model, and multiscale techniques for the lens capsule have demonstrated the ability to match experimental data and other models of lens capsule tissue stretching.

4 Reportable Outcomes

1. Radhakrishnan, S., *Ultrastructural Identification of the Internal Fiber Cells of the Mammalian Ocular Lens*, Senior Thesis, Department of Chemical and Biological Engineering, University of Colorado Boulder, April 2014.
2. Regueiro, R.A., Zhang, B., Wozniak, S.L. (2014) Large deformation dynamic three-dimensional coupled finite element analysis of soft biological tissues treated as biphasic porous media, *Comp. Model. Eng. Sci.*, 98(1):1-39.

5 Conclusion

The research progress over the last year has focussed on imaging of the lens fiber cell structure using confocal laser scanning microscopy (CLSM), and type IV collagen structure using cryo-electron microscopy and tomography (cryo-EM and ET). The CLSM promises to generate good structural identification of lens fiber cells, while the cryo-EM and ET have not yet positively identified type IV collagen structure of the lens capsule.

The collaborations with Dr. Vernerey of UCB and Dr. Burd of the University of Oxford provide meaningful steps toward developing a multiscale computational model of the ocular lens being able to simulate large deformations under traumatic loading.

References

M.R. Banitt, J.B. Malta, S.L. Mian, and H.K. Soong. Rupture of anterior lens capsule from blunt ocular injury. *Journal of Cataract and Refractive Surgery*, 35(5):943–945, MAY 2009. ISSN 0886-3350. doi: 10.1016/j.jcrs.2008.11.066.

K. Barnard, S.A. Burgess, D.A. Carter, and D.M. Woolley. Three-dimensional structure of type IV collagen in the mammalian lens capsule. *J. Struct. Biol.*, 108(1):6–13, 1992.

H.J. Burd. A structural constitutive model for the human lens capsule. *Biomechanics and Modeling in Mechanobiology*, 8(3):217–231, 2009.

S. Krag and T.T. Andreassen. Biomechanical measurements of the porcine lens capsule. *Exp. Eye Res.*, 62(3):253–260, 1996. ISSN 0014-4835.

T.H. Mader, J.V. Aragones, A.C. Chanlder, J.A. Hazlehurst, J. Heier, J.D. Kingham, and E. Stein. Ocular and ocular adnexal injuries treated by United States military ophthalmologists during operations Desert Shield and Desert Storm. *Ophthalmology*, 100(10):1462–1467, OCT 1993.

T.H. Mader, R.D. Carroll, C.S. Slade, R.K. George, J.P. Ritchey, and S.P. Neville. Ocular war injuries of the Iraqi insurgency, January-September 2004. *Opthalmology*, 113(1):97–104, JAN 2006.

L.M. Parver, A.L. Dannenberg, B. Blacklow, C.J. Fowler, R.J. Brechner, and J.M. Tielsch. Characteristics and causes of penetrating eye injuries reported to the National Eye Trauma System Registry, 1985-91. *Public Health Reports*, 108(5):625–632, SEP-OCT 1993.

W.L. Walter. Intralenticular foreign body - as a cause of traumatic cataract. *American Journal of Ophthalmology*, 54(5):856–&, 1962.

E.D. Weichel and M.H. Colyer. Combat ocular trauma and systemic injury. *Current Opinion in Ophthalmology*, 19(6):519–525, NOV 2008. ISSN 1040-8738. doi: 10.1097/ICU.0b013e3283140e98.

T.Y. Wong, M.B. Seet, and C.L. Ang. Eye injuries in twentieth century warfare: A historical perspective. *Survey of Ophthalmology*, 41(6):433–459, MAY-JUN 1997. ISSN 0039-6257.